AMENDMENTS TO THE SPECIFICATION

1) Please amend the specification as indicated in the marked-up paragraphs below. The numbers through [0007] correspond to the numbering in the application as filed, as well as the numbering of the published application. For subsequent paragraphs, the published application is cross referenced to the application as filed.

[0002] In view of economic viability, processes for the production of pharmaceutically relevant proteins must lead to biologically active products of the highest possible purity. The expression of such relevant proteins in yeasts is widely used for this purpose. The production of proteins such as insulin, GM-CSF (~~Leukine~~ LEUKINE®) and hirudin derivatives like lepirudin (~~Refludan~~ REFLUDAN®), is an example of the successful development of genetic engineering processes which are based on the synthesis of the particular protein or precursors thereof in yeast. Generally, yeasts can directly synthesize hirudins with good yields which are on the gram scale when using Hansenula polymorpha (Weydemann et al., Appl. Microbiol Biotechnol. 44: 377-385, 1995) or Pichia pastoris (Rosenfeld et al., Protein Expr. Purif: 4, 476 -82, 1996).

[0003] EP-A 0 324 712 describes a hirudin derivative (~~Refludan~~ REFLUDAN®) whose N-terminal amino acid is leucine and its constitutive expression in Saccharomyces cerevisiae strain Y79. EP-A 0 347 781 describes mini-proinsulin and, by way of example, its expression in bakers' yeast. ~~Refludan~~ REFLUDAN®. and insulin are produced by carrying out two separate expressions.

[0007] However, the production of a plurality of products is optional. The amount of needed ~~Refludan~~ REFLUDAN®, for example, is less than that of insulin, and this may result in processes in which one of the pharmaceutically interesting substances is discarded.

2) Please replace published paragraph [0061], originally at page 14, the title corresponding to EXAMPLE 1, as indicated below:

Construction of a Yeast Expression Plasmid Encoding Hirudin (~~Refludan~~ REFLUDAN®)-Lys-Arg-mini-proinsulin

3) Please replace published paragraph [0062], originally at page 14, [043], as indicated below:

[0062] Starting materials were the plasmids pK152 (PCT/EP00/08537, which is incorporated by reference herein in its entirety), pSW3 (EP-A 0 347 781, which is incorporated by reference herein in its entirety) and the recombinant yeast plasmid derivative coding for bovine interleukin 2, which was pαADH2 plus the cDNA for IL2 (Price et al., Gene 55, 1987, which is incorporated by reference herein in its entirety). The yeast plasmid was distinguished by the fact that it carries the α factor leader sequence under the control of the yeast ADH2 promoter. This sequence was followed by the bovine interleukin 2 cDNA sequence which was connected via a Kpnl restriction enzyme recognition site and which contained an Ncol restriction enzyme recognition site in the untranslated 3' end which was unique in the vector. Thus, the cDNA sequence was readily removable from the plasmid via Kpnl/Ncol cleavage. Since good expression yields were reported, it was assumed that the remaining 3' interleukin 2 sequence (as a terminator sequence) had a stabilizing effect on the mRNA and thus need not be deleted or replaced by a yeast terminator sequence. Plasmid pK152 carried the DNA sequence coding for Leu-hirudin (~~Refludan~~ REFLUDAN®) and plasmid pSW3 carried the DNA sequence for mini-proinsulin. The gene sequence which was to encode hirudin-Lys Arg-mini-proinsulin was first prepared by means of PCR technology. For this purpose, 4 primers were prepared with the aid of the ~~Expedite~~ EXPEDITE™ DNA synthesis system:

1 i. hir_insfkr (SEQ ID NO:1, encoded protein segment: SEQ ID NO:2)

I P E E Y L Q K R F V N Q H L C

5'-ATCCCTGAGGAATACCTTCAGAAGCGATTTGTTAACCAACACTTGTGTGG-3'

59 60 61 62 63 64 65 B1 B2 B3 B4 B5 B6 B7

ii. hir_in srevkr (SEQ ID NO:3)

5'-CCTCACAAGTG TTGGTTAACA AATCGCTTCT GAAGGTATTC CTCAGGAT-3'

iii. hirf1 (SEQ ID NO:4, encoded protein segment: SEQ ID NO:5)

L T Y T D C

5'-TTTTTTTGGATCCTTTGGATAAAAGACTTACGTATACTGACTGCAC

iv. insncol rev (SEQ ID NO:6)

5'-TTTTTTCCAT GGGTCGACTATCAG-3'

4) Please replace published paragraph [0065], originally at page 17, the title corresponding to EXAMPLE 2, as indicated below:

Construction of a Yeast Expression Plasmid Encoding Hirudin (~~Refludan~~ REFLUDAN)-Lys-Arg-insulin B chain-Lys-Arg-insulin A chain

5) Please replace published paragraph [0085], originally at [064], as indicated below:

The hirudin concentration was determined according to the method of GrieRbach et al. (Thrombosis Research 37, pp. 347-350, 1985, which is incorporated by reference herein in its entirety). For this purpose, ~~Refludan~~ REFLUDAN®. standard was included in the measurements in order to establish a calibration curve from which the yield in mg/l was determined directly.

6) Please replace published paragraph [0087], originally at [065], as indicated below:

~~Invitrogen~~ INVITROGEN®. sells a cloning and expression kit for preparing recombinant proteins using *P. pastoris* as a host system. For this, a detailed technical protocol regarding preparation and subsequent expression of a *P. pastoris* system for the production of a desired recombinant protein is provided so that only the construction of the expression vector encoding the desired protein has to be described when following said protocols. The ~~EasySelect~~ EASYSELECT™ Pichia expression kit (catalog no. K1740-01) was used.

7) Please replace published paragraph [0091], originally at [069], as indicated below:

The purification requires separation of the two proteins of the fusion protein at an early stage. The fusion protein is processed to ~~Refludan~~ REFLUDAN® -LysArg and mini-proinsulin by the natural yeast protease system. After completion of the expression of Example 6, the medium is analyzed by means of analytical RP-HPLC. In contrast to most other polypeptides found in the supernatant due to either spontaneous lysis of yeast cells or secretion, the two proteins, hirudin and mini-proinsulin, are not precipitated at pH 2.5-3. The culture medium is therefore acidified appropriately, using concentrated H_2SO_4, to pH 2.5-3.5 and then, after completion of the precipitation which takes at least 2 hours, the precipitate and the cells are removed by centrifugation. After centrifugation, the medium is adjusted using NaOH to pH 3.5-7 and the two components hirudin and mini-proinsulin are separated from one another by means of hydrophobic interaction chromatography, for example by using a chromatography column filled with Diaion HP20® material as described in EP-A 0 347 781, which is incorporated by reference herein in its entirety. Hirudin can then be isolated from the hirudin-containing fractions according to EP-A 0 549 915, which is incorporated by reference herein in its entirety, and insulin can be isolated from the mini-proinsulin-containing fractions according to EP-A 0 347 781, which is incorporated by reference herein in its entirety.

8) Please replace published paragraph [0093], originally at [070], as indicated below:

At the end of the expression period, the culture medium is adjusted using concentrated H_2SO_4 to pH 6.8 and trypsin is then added with stirring so that a final concentration of 4-8 mg per liter is established. After incubation for approximately 4 hours, the fermentation broth treated in this way is adjusted using concentrated H_2SO_4 to pH 2.5-3. After 1-6 hours of precipitation, the precipitate is removed by centrifugation at greater than 5000 X g. The mono-Arg-insulin formed is then isolated via ion exchange chromatography, by ~~S-Sepharose~~ S-SEPHAROSE® in a buffer of 50 mM lactic acid and 30% (v/v) isopropanol (pH 3.5). Elution is carried out by means of an NaCl linear gradient of 0.05-0.5 M salt. The product-containing fractions are diluted 1:1 with H_2O and then $ZnCl_2$ is added, so that a 0.1% strength $ZnCl_2$ solution is formed. In this regard, the fractions are analyzed for insulin by SDS-PAGE analysis and by Western Blot analysis. For standard Western Blot experiments the polyclonal Guinea Pig Anti-insulin (Code NO.:A0564, DAKO Corp.) is used. Mono-Arg-insulin precipitates at pH 6.8 and is converted to insulin according to EP-A 0 324 712, which is incorporated by reference herein in its entirety.